

Effects of Planting Row Spacing and Cultivar on Denitrifying Bacterial Community Abundance and Function in the Maize Rhizosphere (Postprint)

Authors: He Song, Jin Wenjun, Che Zhao, Cao Wenchao, Zhaorong Dong, Wang Chengyu

Date: 2017-11-09T00:00:00+00:00

Abstract

Denitrification is an important pathway for nitrogen loss in the rhizosphere, and it remains unclear whether crop varieties and row spacing alterations affect rhizosphere denitrification. This study compared differences in the abundance and function of denitrifying microbial communities in rhizosphere soils among different maize varieties and planting row spacings, providing a scientific basis for reducing rhizosphere denitrification losses and improving nitrogen use efficiency. Through two independent field experiments and using biochemical and molecular microbiological methods, we investigated the effects of five maize varieties (‘Jundan 20’, ‘Annong 8’, ‘Zhengdan 958’, ‘Pinyu 18’, and ‘Longping 206’) and four planting row spacings (20 cm, 30 cm, 40 cm, and 50 cm) on rhizosphere soil denitrification capacity, denitrifier abundance, $N_2O/(N_2O+N_2)$ product ratio, and soil respiration. The rhizosphere denitrification capacities of ‘Jundan 20’, ‘Annong 8’, and ‘Zhengdan 958’ were significantly lower than those of the other two varieties; as row spacing decreased, denitrification capacity showed a significant increasing trend. The abundance of nirS-type denitrifiers in ‘Longping 206’ and ‘Pinyu 18’ was significantly higher than in other varieties, whereas the abundances of nirK- and nosZ-type bacteria were highest in ‘Jundan 20’ and ‘Annong 8’. The abundances of nirS- and nirK-type bacteria at 20 cm row spacing were significantly higher than in other row spacing treatments, but nosZ-type bacterial abundance was greatest at 40 cm row spacing. Variety had a certain effect on the $N_2O/(N_2O+N_2)$ product ratio, with ‘Annong 8’ showing the lowest ratio, but row spacing had no significant effect on the product ratio. Correlation analysis indicated that denitrification capacity was extremely significantly positively correlated with both soil respiration and nirS-type bacterial abundance, but did not exhibit such relationships with nosZ- and nirK-type bacteria,

suggesting that nirS-type bacterial abundance and differences in rhizosphere organic carbon may be the dominant factors causing variations in denitrification capacity. Both variety and planting row spacing exert certain influences on the maize rhizosphere denitrification process; screening, selecting, and breeding low-denitrification-loss varieties and regulating the rhizosphere denitrification process are effective approaches for reducing rhizosphere denitrification losses and improving nitrogen use efficiency.

Full Text

Preamble

Effects of Row Spacing and Maize Variety on Abundance and Function of Denitrifying Bacterial Flora in Maize Rhizosphere

SONG He¹, JIN Wenjun¹, CHE Zhao¹, CAO Wenchao², DONG Zhaorong¹, WANG Chengyu^{1**}

(1. College of Agronomy, Anhui Agricultural University, Hefei 230036, China; 2. College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China)

Abstract: Denitrification is a key pathway of nitrogen loss in the rhizosphere, yet it remains unclear whether changes in crop variety and row spacing affect rhizosphere denitrification. This study compared differences in the abundance and function of denitrifying bacterial communities among different maize varieties and row spacings to provide a scientific basis for reducing rhizosphere denitrification losses and improving nitrogen use efficiency. Using biochemical and molecular microbiological methods in two independent field experiments, we investigated the effects of five maize varieties (‘Xundan 20’ , ‘Annong 8’ , ‘Zhengdan 958’ , ‘Pinyu 18’ , and ‘Longping 206’) and four row spacings (20 cm, 30 cm, 40 cm, and 50 cm) on rhizosphere denitrification capacity, denitrifier abundance, $N_2O/(N_2O+N_2)$ product ratio, and soil respiration. Denitrification capacities in the rhizospheres of ‘Xundan 20’ , ‘Annong 8’ , and ‘Zhengdan 958’ were significantly lower than those of the other two varieties, and denitrification capacity showed a significant increasing trend as row spacing decreased. The abundances of nirS-type denitrifiers in the rhizospheres of ‘Longping 206’ and ‘Pinyu 18’ were significantly higher than in other varieties, whereas nirK- and nosZ-type denitrifier abundances were highest for ‘Xundan 20’ and ‘Annong 8’ . The 20 cm row spacing treatment showed significantly higher abundances of nirS- and nirK-type denitrifiers compared to other row spacings, but nosZ-type denitrifier abundance was greatest at 40 cm row spacing. Variety had a certain influence on the $N_2O/(N_2O+N_2)$ product ratio, with ‘Annong 8’ showing the lowest ratio, but row spacing had no significant effect on the product ratio. Correlation analysis revealed that denitrification capacity was extremely significantly positively correlated with both soil respiration and nirS-type denitrifier abundance, but showed no such relationship with nosZ- or nirK-type

denitrifiers, suggesting that differences in nirS-type denitrifier abundance and rhizosphere organic carbon were the dominant factors causing variation in denitrification capacity. Both variety and planting row spacing influence maize rhizosphere denitrification processes, and screening/breeding varieties with low denitrification losses combined with regulation of rhizosphere denitrification processes represent effective approaches for reducing rhizosphere nitrogen loss and improving nitrogen use efficiency.

Keywords: Maize; Variety; Row spacing; Rhizosphere; Denitrification capacity; Denitrifying bacterial flora; $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ ratio

The rhizosphere serves as the central hub for plant-soil-microbe interactions and represents the most active zone for material exchange between plants and the soil ecosystem [1]. Regulation of rhizosphere ecological processes is key to improving crop nutrient use efficiency. Nitrogen transformation is one of the most critical ecological processes in the rhizosphere, closely related to both crop nitrogen use efficiency and nitrogen-associated environmental pollution. During nitrogen transformation, the denitrification process reduces plant-available NO_3^- to gaseous products such as N_2O , NO , and N_2 that are difficult for plants to utilize, directly causing nitrogen loss from the rhizosphere. Furthermore, N_2O and NO are major trace gases in the atmosphere that play important roles in global warming and ozone layer depletion [2,3]. Therefore, in-depth investigation of rhizosphere soil denitrification processes is of great significance for improving crop nitrogen use efficiency, reducing trace gas emissions, and promoting low-carbon agriculture.

Denitrification processes are primarily driven by denitrifying bacterial communities and are regulated by factors such as soil NO_3^- concentration, pH, organic carbon content, and salinity [4,5]. During crop growth, roots influence rhizosphere denitrification by absorbing NO_3^- and alkaline ions or releasing organic carbon, thereby altering rhizosphere soil NO_3^- concentration, pH, salinity, and organic carbon content. For example, crops can release approximately 17% of total photosynthates through their roots during their lifespan, providing an important energy source for rhizosphere denitrifiers [6]. In some soils with high NO_3^- content, rhizosphere denitrification rates can be 22 times higher than in bulk soil [7]. Different plant species exhibit differences in nutrient uptake and organic carbon secretion, which should result in different rhizosphere denitrification characteristics. Previous studies have found that different plant species significantly affect the community structure of nirK-type denitrifiers in the rhizosphere [8]. Similarly, different genotypes of the same crop also show variations in the quantity and quality of nutrient uptake and organic carbon secretion [9-11], but whether these differences affect denitrifier communities and their functions remains unclear. In addition to variety selection, adjusting row spacing to optimize plant population density is an effective measure for crop yield improvement that is widely applied in agricultural production. Different planting row spacings significantly affect crop root nutrient uptake and organic matter

release [12,13], but the impact of row spacing on rhizosphere denitrification is also unclear.

Through two independent field plot experiments, this study compared differences in the abundances of nirS-, nirK-, and nosZ-type denitrifiers in rhizosphere soil among different maize varieties and planting row spacings. Combined with measurements of denitrification capacity and $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratios, we aimed to clarify the functional changes in denitrification and provide a scientific basis for understanding differences in rhizosphere denitrification characteristics among varieties and row spacings, reducing rhizosphere nitrogen loss, and improving crop nitrogen use efficiency.

1.1 Experimental Site Description

This study included two experimental sites: Funan and Hefei. The Funan site was located at the experimental farm of Funan County Agricultural Science Research Institute in Anhui Province (32°66' N, 115°59' E). The region has an average annual temperature of 15 °C and annual precipitation of 900 mm. The soil type is lime concretion black soil. Before the experiment, the surface soil (0-20 cm) contained 1.53 mg · kg⁻¹ total nitrogen, 83.2 mg · kg⁻¹ alkaline-hydrolyzable nitrogen, 56.5 mg · kg⁻¹ available phosphorus, 78.7 mg · kg⁻¹ available potassium, and 16.8 g · kg⁻¹ organic matter [14]. The Hefei site was located at the Nongcuiyuan Experimental Base of Anhui Agricultural University in Hefei City, Anhui Province (31°87' N, 117°25' E). This region has an average annual temperature of 15.7 °C and annual precipitation of nearly 1000 mm. The soil type is yellow-cinnamon soil. Before maize planting, the 0-20 cm soil layer contained 14.5 g · kg⁻¹ organic matter, 1.21 mg · kg⁻¹ total nitrogen, 76.4 mg · kg⁻¹ alkaline-hydrolyzable nitrogen, 32.4 mg · kg⁻¹ available phosphorus, and 84.6 mg · kg⁻¹ available potassium.

1.2 Experimental Design and Sample Collection

Experiment 1 was a maize variety comparison trial conducted at the Funan site, while Experiment 2 examined different planting row spacings at the Hefei site. Both experiments employed a completely randomized design with three replicates per treatment. In Experiment 1, each plot covered 28 m² (4 m × 7 m). The tested maize varieties included 'Xundan 20' (XD), 'Annong 8' (AN), 'Zhengdan 958' (ZD), 'Pinyu 18' (PY), and 'Longping 206' (LP). Sowing occurred on June 10, 2013, with a planting density of 60,000 plants · hm⁻² and nitrogen application of 240 kg(N) · hm⁻². The basal-to-topdressing ratio was 5:5, with topdressing applied at the large trumpet stage. Phosphorus and potassium fertilizers were applied at 105 kg(P O) · hm⁻² and 105 kg(K O) · hm⁻², respectively, all as basal fertilizers. Additional experimental details can be found in reference [14].

In Experiment 2, the maize variety was 'Zhengdan 958'. Each plot covered 8 m² (2 m × 4 m) with four row spacing treatments of 20 cm, 30 cm, 40 cm, and

50 cm, all with a plant spacing of 30 cm. Sowing occurred on June 16, 2013, with nitrogen application of $220 \text{ kg(N)} \cdot \text{hm}^{-2}$. The basal-to-topdressing ratio was 5:5, with topdressing applied at the large trumpet stage. Phosphorus and potassium fertilizers were applied at $120 \text{ kg(P O)} \cdot \text{hm}^{-2}$ and $110 \text{ kg(K O)} \cdot \text{hm}^{-2}$, respectively, all as basal fertilizers.

At the maize flowering stage, sampling points were randomly selected in each plot. Maize roots were excavated and rhizosphere soil was collected using the shake-root separation method to obtain soil adhering to roots. Bulk soil was collected from between rows using a 5 cm diameter soil auger [15]. Samples were placed in plastic bags, stored in a cooler with ice packs, transported to the laboratory, passed through a 2 mm sieve, and divided into two portions. One portion was stored at $-20 \text{ }^{\circ}\text{C}$ for molecular quantification of denitrification functional genes, while the other was stored at $4 \text{ }^{\circ}\text{C}$ for incubation experiments and analysis of soil physicochemical properties.

1.3 Soil Microbial DNA Extraction and Functional Gene Quantitative PCR

Total soil microbial DNA was extracted from 0.5 g soil samples using a soil DNA extraction kit (Fast DNA SPIN Kit for Soil, Q-BIO gene, USA) following the manufacturer's instructions, with a total elution volume of 80 μL . The eluent was stored at $-20 \text{ }^{\circ}\text{C}$. Three primer pairs were used to amplify denitrification functional genes nirS, nirK, and nosZ: nirScd3aF/nirSR3cd, nirK1F/nirK5R, and nosZ1F/nosZ1R [16-18]. The 50 μL reaction system contained 25 μL SYBR Premix Ex Taq (TaKaRa, Japan), 1 μL of each primer ($10 \text{ }^{\circ}\text{mol} \cdot \text{L}^{-1}$), 1 μL DNA template, and sterile high-purity water to volume. The nirS amplification program was: $95 \text{ }^{\circ}\text{C}$ for 2 min; 35 cycles of $95 \text{ }^{\circ}\text{C}$ for 30 s, $58 \text{ }^{\circ}\text{C}$ for 45 s, and $72 \text{ }^{\circ}\text{C}$ for 45 s. The nirK PCR program was: $94 \text{ }^{\circ}\text{C}$ for 3 min; 35 cycles of $94 \text{ }^{\circ}\text{C}$ for 45 s, $55 \text{ }^{\circ}\text{C}$ for 45 s, and $72 \text{ }^{\circ}\text{C}$ for 1 min. The nosZ PCR program was: $95 \text{ }^{\circ}\text{C}$ for 3 min; 35 cycles of $94 \text{ }^{\circ}\text{C}$ for 20 s, $59 \text{ }^{\circ}\text{C}$ for 20 s, and $72 \text{ }^{\circ}\text{C}$ for 30 s. Quantitative PCR was performed on an Mx3000P real-time PCR system (Stratagene, USA). Plasmid and standard curve preparation followed the method of Wang Xiaohui [19].

1.4 Measurement of Denitrification Capacity (DC), $\text{N O}/(\text{N O}+\text{N})$ Ratio, and Soil Respiration

Denitrification capacity was measured according to the standard method of Šimek [20]. Two sets of 10 g fresh rhizosphere soil from each plot were placed in 120 mL incubation bottles. Four milliliters of $10 \text{ mmol} \cdot \text{L}^{-1}$ KNO_3 solution was added to each bottle, which was then sealed and flushed four times with helium gas. Acetylene (10 mL) was injected into one set of bottles, while the other set received no acetylene. For bottles receiving acetylene, a syringe containing a small amount of water without a plunger was inserted through the stopper to balance internal pressure. After 24 h and 48 h, 5 mL gas samples were withdrawn from both sets for measurement of N O and CO_2 concentrations by gas

chromatography (Agilent GC-7890A, USA). To prevent pressure reduction, an equivalent volume of helium was added to maintain pressure balance. Denitrification capacity, $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ ratio, and soil respiration were calculated based on the rate of change in N_2O and CO_2 concentrations between 24 h and 48 h [20,21]. The N_2O gas change rate in acetylene-amended bottles represented denitrification capacity, indicating the total production rate of $\text{N}_2\text{O}+\text{N}_2$, while N_2O gas change in bottles without acetylene represented the N_2O emission rate from denitrification.

1.5 Measurement of Soil pH, Inorganic Nitrogen, Electrical Conductivity, and Water Content

Soil inorganic nitrogen (NH_4^+-N and NO_3^--N) was extracted with $1.0 \text{ mol} \cdot \text{L}^{-1}$ KCl and measured using a flow analyzer (TRAACS2000, Hamburg, Germany). Electrical conductivity and soil pH were measured with a conductivity meter and pH meter (Leici, Shanghai) at a soil-to-water ratio of 5:1. Soil water content (%) was calculated as soil water mass divided by oven-dried soil mass multiplied by 100%.

1.6 Data Statistical Analysis

Data were analyzed using SPSS 19 software for one-way ANOVA and correlation analysis. Values in figures represent treatment means \pm standard deviation. Figures were created using Microsoft Excel 2007.

2.1 Effects of Maize Variety and Row Spacing on Rhizosphere Soil Chemical Properties

As shown in Table 1, distinct differences existed in basic chemical properties among rhizosphere soils of different maize varieties. The rhizosphere soil pH of 'Longping 206' was significantly lower than other varieties. Nitrate nitrogen content was highest in 'Longping 206' and lowest in 'Xundan 20', whereas ammonium nitrogen content showed the opposite pattern. Electrical conductivity was highest in 'Annong 8' and 'Pinyu 18', intermediate in 'Zhengdan 958' and 'Longping 206', and lowest in 'Xundan 20'. Water content also varied among varieties, with 'Xundan 20' and 'Longping 206' significantly higher than 'Annong 8' and 'Pinyu 18'.

Planting row spacing also significantly affected soil chemical properties. As row spacing decreased, rhizosphere soil pH significantly declined, reaching 5.55 at 20 cm row spacing—nearly one unit lower than bulk soil. For inorganic nitrogen, the 50 cm row spacing treatment was significantly lower than other treatments. Soil electrical conductivity was highest at 40 cm row spacing. Additionally, both variety and row spacing experiments showed that rhizosphere soil pH at the maize flowering stage was significantly lower than surrounding bulk soil.

2.2 Effects of Maize Variety and Row Spacing on Rhizosphere Denitrifier Abundance

Figure 1 [Figure 1: see original paper]A shows that nirS-type denitrifier abundances in the rhizospheres of ‘Pinyu 18’ and ‘Longping 206’ were significantly higher than in the other three maize varieties. For nirK- and nosZ-type denitrifiers, ‘Xundan 20’ and ‘Annong 8’ showed significantly higher abundances than the other three varieties (Figure 1). Furthermore, the nosZ/(nirS+nirK) ratio in ‘Xundan 20’, ‘Annong 8’, and ‘Zhengdan 958’ was significantly higher than in ‘Pinyu 18’ and ‘Longping 206’ (Figure 1B).

Row spacing also significantly affected denitrifier communities. As shown in Figure 2 [Figure 2: see original paper]A, the 20 cm row spacing treatment exhibited significantly higher abundances of nirS- and nirK-type denitrifiers in rhizosphere soil compared to the other three row spacing treatments. For nosZ-type denitrifiers, abundance was highest at 40 cm row spacing, with no significant differences among other treatments (Figure 2B). Unlike variety effects, row spacing had minimal impact on the nosZ/(nirS+nirK) ratio, with no significant differences among treatments (Figure 2B).

Comparison of rhizosphere and bulk soils across both experiments revealed that nirS-type denitrifier abundance was significantly higher in rhizosphere soil, whereas nirK- and nosZ-type denitrifier abundances were not consistently higher in rhizosphere soil (Figures 1 and 2). In both experiments, nirS-type denitrifier abundance in rhizosphere soil was at least two orders of magnitude higher than nirK-type abundance (Figures 1 and 2).

2.3 Effects of Maize Variety and Row Spacing on Rhizosphere Denitrification Capacity, $\text{NO}/(\text{NO}+\text{N}_2\text{O})$ Ratio, and Soil Respiration

As shown in Figure 3 [Figure 3: see original paper]A, rhizosphere soil denitrification capacity in ‘Longping 206’ and ‘Pinyu 18’ was significantly higher than in the other three varieties. Both the $\text{NO}/(\text{NO}+\text{N}_2\text{O})$ product ratio and rhizosphere soil respiration in ‘Annong 8’ were significantly lower than other varieties (Figure 3). In addition to variety effects, row spacing significantly influenced denitrification capacity and soil respiration. Denitrification capacity showed a significant increasing trend as row spacing decreased, with the highest value at 20 cm spacing (Figure 4 [Figure 4: see original paper]A). Soil respiration at 20 cm row spacing was also significantly higher than in treatments with wider spacing. Unlike variety effects, row spacing had no significant influence on the $\text{NO}/(\text{NO}+\text{N}_2\text{O})$ product ratio. In both experiments, rhizosphere soil denitrification capacity and soil respiration were significantly higher than in bulk soil.

2.4 Correlation Analysis Among Measured Variables in Variety and Row Spacing Experiments

In both experiments, denitrification capacity was extremely significantly positively correlated with soil respiration ($r = 0.889$ and 0.866 , $P < 0.01$) and extremely significantly negatively correlated with soil pH ($r = -0.726$ and -0.918 , $P < 0.001$). Soil respiration also showed an extremely significant negative correlation with soil pH. Additionally, denitrification capacity was extremely significantly positively correlated with nirS-type denitrifier abundance ($r = 0.850$ and 0.795 , $P < 0.01$). In the variety comparison experiment, denitrification capacity was extremely significantly negatively correlated with nirK-type denitrifier abundance, but this correlation was not significant in the row spacing experiment (Tables 2 and 3). In both experiments, denitrification capacity was not correlated with nosZ-type denitrifier abundance. Regarding the $\text{NO}/(\text{NO} + \text{N}_2\text{O})$ product ratio, it was extremely significantly negatively correlated with $\text{nosZ}/(\text{nirK} + \text{nirS})$ in the variety comparison experiment ($r = -0.701$, $P = 0.001$), but not correlated with nosZ-type denitrifier abundance. In the row spacing experiment, the product ratio was not correlated with either $\text{nosZ}/(\text{nirK} + \text{nirS})$ or nosZ-type denitrifier abundance.

Rhizosphere soil denitrification processes are closely related to rhizosphere nitrogen loss and crop nitrogen use efficiency [22]. This study investigated the effects of variety and planting row spacing on rhizosphere denitrification processes. The results demonstrated significant differences in rhizosphere soil denitrification capacity among maize varieties. ‘Xundan 20’, ‘Annong 8’, and ‘Zhengdan 958’ showed significantly lower rhizosphere soil denitrification capacity than ‘Longping 206’ and ‘Pinyu 18’, indicating relatively less rhizosphere nitrogen loss through denitrification in the first three varieties. Current approaches for improving crop nitrogen use efficiency primarily focus on precision fertilization [23] and breeding varieties with high nitrogen uptake capacity [24], with little consideration given to rhizosphere nitrogen loss, particularly through denitrification. In reality, reducing rhizosphere nitrogen denitrification loss may represent another important pathway for improving crop nitrogen use efficiency. Future research should emphasize the regulation of rhizosphere soil denitrification processes and the application of low-denitrification-loss varieties.

Among current crop cultivation practices, “increasing density while reducing nitrogen” is an important strategy for improving nitrogen use efficiency and reducing negative environmental effects of nitrogen fertilizer [25]. For example, Al-Naggar et al. [26] found that for some density-tolerant maize varieties, when planting density increased from 71,400 to 95,200 plants per hectare, nitrogen application could be reduced from 570 to 285 kg N per hectare while maintaining similar yields. However, this study showed that as planting row spacing decreased (i.e., planting density increased), maize rhizosphere soil denitrification capacity continuously increased. This suggests that increasing crop density should be controlled within certain limits. If a critical density threshold is exceeded, rhizosphere nitrogen denitrification loss may increase substantially,

necessitating higher nitrogen inputs to achieve high yields. Under such conditions, the effectiveness of the “increase density, reduce nitrogen” strategy would be greatly diminished, making it difficult to achieve nitrogen reduction goals. Boomsma et al. [27] demonstrated that when planting density increased from 79,000 to 104,000 plants per hectare, nitrogen input actually needed to increase by 165 kg N to achieve the same yield.

Among the measured indicators, rhizosphere denitrification capacity was extremely significantly positively correlated with soil respiration in both experiments. Soil respiration represents soil organic carbon mineralization [20] and reflects the amount of microbially available organic carbon in soil [28]. We therefore hypothesize that the effects of maize variety and planting row spacing on rhizosphere soil denitrification processes are largely caused by differences in rhizosphere organic carbon secretion. Organic acids are important components of rhizosphere exudates, and their secretion is significantly negatively correlated with rhizosphere soil pH [29]. This study also found an extremely significant negative correlation between rhizosphere soil pH and soil respiration. Thus, significant differences in rhizosphere soil pH observed in both variety and row spacing experiments provide some evidence for variation in rhizosphere organic carbon secretion. Denitrifying bacteria are mainly heterotrophic microorganisms that use organic carbon as an energy source. Differences in rhizosphere organic carbon supply will consequently affect denitrification capacity. Therefore, in future scientific practice, controlling rhizosphere soil nitrogen denitrification loss should first focus on regulating plant root organic matter secretion.

In addition to environmental factors, denitrifier communities directly participate in rhizosphere denitrification processes, and changes in their abundance may also affect denitrification capacity. This study showed that different maize varieties and planting row spacings affected *nosZ*-, *nirS*-, and *nirK*-type denitrifiers, but their abundances showed different relationships with soil denitrification capacity. In both experiments, *nosZ*-type denitrifier abundance was not significantly correlated with denitrification capacity, whereas *nirS*-type denitrifier abundance was extremely significantly positively correlated with denitrification capacity. *nirK*-type denitrifier abundance was negatively correlated with denitrification capacity in the variety experiment, but this correlation was not significant in the row spacing experiment. Research indicates that *nosZ*-type bacteria participate in the terminal reduction of NO and are more related to the $\text{NO}/(\text{NO}+\text{N}_2)$ product ratio than to denitrification capacity [30]. Both *nirS*- and *nirK*-type denitrifiers participate in the second step of denitrification (NO reduction), and their different relationships with denitrification capacity may reflect distinct ecological niches. Both experiments showed that *nirS*-type denitrifier abundance in maize rhizosphere soil was at least two orders of magnitude higher than *nirK*-type abundance, indicating that *nirS*-type bacteria play an absolutely dominant role in NO reduction in maize rhizosphere, while intense competition from *nirS* bacteria results in poor or even negative correlations between *nirK*-type abundance and rhizosphere denitrification capacity. The weak competitive ability of *nirK*-type bacteria in rhizosphere soil may be influenced by two main

factors. First, compared to nirS-type denitrifiers, nirK-type bacteria are more strongly affected by pH reduction [31]. This study showed that rhizosphere soil pH was significantly lower than bulk soil, with some treatments decreasing by nearly one unit. Additionally, nirS-type denitrifiers prefer environments with higher organic carbon and nutrient contents compared to nirK-type bacteria, and rhizosphere soil precisely exhibits these characteristics [32].

In addition to denitrification capacity, the $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratio has attracted considerable attention due to its close relationship with trace gas N_2O emissions. The N_2O reductase encoded by the *nosZ* gene is considered the only biological sink for N_2O , so *nosZ*-type denitrifier abundance is generally thought to be strongly negatively correlated with the $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratio [33]. However, results from both experiments in this study showed no significant correlation between *nosZ* abundance and the $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratio. Recent studies [34,35] suggest that compared to absolute *nosZ* abundance, the *nosZ*/*(nirS+nirK)* abundance ratio is more comparable to the $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratio because the *nosZ*/*(nirS+nirK)* ratio considers both N_2O reduction and production, better reflecting the “source-sink balance” of N_2O . In the variety experiment of this study, the *nosZ*/*(nirS+nirK)* ratio was extremely significantly negatively correlated with the $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratio, but this correlation was not significant in the row spacing experiment. This discrepancy may be primarily related to large differences in the *nosZ*/*(nirS+nirK)* ratio between the two experiments. In the variety comparison experiment, the *nosZ*/*(nirS+nirK)* ratio ranged from 4.3% to 35.2%, with *nosZ*-type bacteria occupying a certain proportion of the denitrifier community and playing a notable role. In contrast, the row spacing comparison experiment showed a *nosZ*/*(nirS+nirK)* ratio of only 0.4% to 1.3%, with *nosZ*-type bacteria representing too small a proportion of the denitrifier population to exert substantial effects, making the relationship with the $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratio susceptible to interference from other factors.

Both variety and planting row spacing significantly affect maize rhizosphere soil denitrification processes. ‘Xundan 20’, ‘Annong 8’, and ‘Zhengdan 958’ showed significantly lower rhizosphere denitrification capacity than the other two varieties. Denitrification capacity increased significantly as row spacing decreased. Differences in rhizosphere soil organic carbon content and nirS-type denitrifier abundance were the dominant factors causing changes in denitrification processes. Variety had some influence on the denitrification $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratio, but row spacing had no significant effect. In future maize variety breeding and high-efficiency cultivation management, screening and breeding varieties with low rhizosphere denitrification loss combined with regulation of rhizosphere soil denitrification processes represent new and effective approaches for reducing rhizosphere nitrogen denitrification loss, decreasing trace gas emissions, and improving nitrogen use efficiency.

References

- [1] Lu Y H, Zhang F S. The advances in rhizosphere microbiology[J]. *Soils*, 2006, 38(2): 113-121
- [2] Bothe H, Ferguson S J, Newton W E. *Biology of the Nitrogen Cycle*[M]. Amsterdam: Elsevier Science, 2007: 1-427
- [3] Ravishankara A R, Daniel J S, Portmann R W. Nitrous oxide (N O): The dominant ozone depleting substance emitted in the 21st century[J]. *Science*, 2009, 326(5949): 123-125
- [4] Philippot L, Hallin S, Schlote M. Ecology of denitrifying prokaryotes in agricultural soil[J]. *Advances in Agronomy*, 2007, 96: 249-305
- [5] Song H, Wang C Y, Chen Q, et al. Effects of long-term amendment of residue on denitrification characteristics and N O emissions in greenhouse soil[J]. *Chinese Journal of Agrometeorology*, 2014, 35(6): 628-634
- [6] Henry S, Texier S, Hallet S, et al. Disentangling the rhizosphere effect on nitrate reducers and denitrifiers: Insight into the role of root exudates[J]. *Environmental Microbiology*, 2008, 10(11): 3082-3092
- [7] Hamonts K, Clough T J, Stewart A, et al. Effect of nitrogen and waterlogging on denitrifier gene abundance, community structure and activity in the rhizosphere of wheat[J]. *FEMS Microbiology Ecology*, 2013, 83(3): 568-584
- [8] Wang H T, Su J Q, Zheng T L, et al. Impacts of vegetation, tidal process, and depth on the activities, abundances, and community compositions of denitrifiers in mangrove sediment[J]. *Applied Microbiology and Biotechnology*, 2014, 98(22): 9375-9387
- [9] Sheng J, Zhang P, Sun G F, et al. Nutrient absorption characteristics and removal from soil with different wheat varieties based on pollution control[J]. *Ecology and Environmental Sciences*, 2015, 24(3): 487-493
- [10] Liu J G, Qian M, Cai G L, et al. Variations between rice cultivars root secretion of organic acids and relationship with plant cadmium uptake[J]. *Environmental Geochemistry and Health*, 2007, 29(3): 189-195
- [11] Li X G, Zhang T L, Wang X X, et al. The composition of root exudates from two different resistant peanut cultivars and their effects on the growth of soil-borne pathogen[J]. *International Journal of Biological Sciences*, 2013, 9(2):
- [12] Jiang X F, Tao H B, Zheng Z F, et al. Effect of spacing allocation on the root system characters and yield of maize[J]. *Journal of Maize Sciences*, 2013, 21(2): 116-121
- [13] Jiang W S, Wang K J, Wu Q P, et al. Effects of narrow plant spacing on root distribution and physiological nitrogen use efficiency in summer maize[J]. *The Crop Journal*, 2013, 1(1):
- [14] Wang C Y, Song H, Hu L H, et al. Screening for cultivars with water-logging resistance through analyzing morphological indexes and photosynthetic characteristics in maize[J]. *Journal of Anhui Agricultural University*, 2014, 41(4): 533-539
- [15] Yun P, Gao X, Chen L, et al. Plant nitrogen utilization and soil nitrogen status in rhizosphere of maize as affected by various nitrogen rates in wheat-maize rotation system[J]. *Plant Nutrition and Fertilizer Science*, 2010, 16(3):

567-574

- [16] Braker G, Fesefeldt A, Witzel K P. Development of PCR primer systems for amplification of nitrite reductase genes (*nirK* and *nirS*) to detect denitrifying bacteria in environmental samples[J]. *Applied and Environmental Microbiology*, 1998, 64(10): 3769-3775
- [17] Throbäck I N, Enwall K, Jarvis A, et al. Reassessing PCR primers targeting *nirS*, *nirK* and *nosZ* genes for community surveys of denitrifying bacteria with DGGE[J]. *FEMS Microbiology Ecology*, 2004, 49(3): 401-417
- [18] Henry S, Bru D, Stres B, et al. Quantitative detection of the *nosZ* gene, encoding nitrous oxide reductase, and comparison of the abundance of 16S rRNA, *narG*, *nirK*, and *nosZ* genes in *Applied and Environmental Microbiology*, 2006, 72(8): 5181-5189
- [19] Wang X H. Effect of biochar on nitrifying and denitrifying communities in greenhouse soils[D]. Beijing: University of Chinese Academy of Sciences, 2013: 27-29
- [20] Šimek M, Kalčík J. Carbon and nitrate utilization in soils: The effect of long-term fertilization on potential denitrification[J]. *Geoderma*, 1998, 83(3/4): 269-280
- [21] Šimek M, Hopkins D W. Regulation of potential denitrification by soil pH in long-term fertilized arable soils[J]. *Biology and Fertility of Soils*, 1999, 30(1/2): 41-47
- [22] Zhou X G, Guan S N, Wu F Z. Composition of soil microbial communities in the rhizosphere of cucumber cultivars with differing nitrogen acquisition efficiency[J]. *Applied Soil Ecology*, 2015, 95: 90-98
- [23] Williams A, Davis A S, Ewing P M, et al. Precision control of soil nitrogen cycling via soil functional zone management[J]. *Agriculture, Ecosystems & Environment*, 2016, 231: 291-295
- [24] Ohunakin A O, Adebisi S O. Nitrogen use and assimilate partitioning ability for grain yield in some selected maize (*Zea mays* L.) inbred lines genotypes[J]. *American Journal of Experimental Agriculture*, 2015, 5(2): 148-155
- [25] Monneveux P, Zaidi P H, Sanchez C. Population density and low nitrogen affects yield-associated traits tropical maize[J]. *Crop Science*, 2005, 45(2): 535-545
- [26] Al-Naggar A M M, Shabana R A, Atta M M M, et al. Maize response to elevated plant density combined with lowered N-fertilizer rate is genotype-dependent[J]. *The Crop Journal*, 2015, 3(2): 96-109
- [27] Boomsma C R, Santini J B, Tollenaar M, et al. Maize morphophysiological responses to intense crowding and low nitrogen availability: An analysis and review[J]. *Agronomy Journal*, 2009, 101(6): 1426-1452
- [28] Wang W J, Dalal R C, Moody P W, et al. Relationships of soil respiration to microbial biomass, substrate availability and clay content[J]. *Soil Biology and Biochemistry*, 2003, 35(2):
- [29] Zeng F R, Chen S, Miao Y, et al. Changes of organic acid exudation and rhizosphere pH in rice plants under chromium stress[J]. *Environmental Pollution*, 2008, 155(2): 284-289
- [30] Raut N, Dörsch P, Sitaula B K, et al. Soil acidification by intensified crop

- production in south Asia results in higher $\text{N}_2\text{O}/(\text{N}_2\text{O}+\text{N}_2)$ product ratios of denitrification[J]. *Soil Biology and Biochemistry*, 2012, 55: 104-112
- [31] Yoshida M, Ishii S, Otsuka S, et al. nirK-harboring denitrifiers are more responsive to denitrification-inducing conditions in rice paddy soil than nirS-harboring bacteria[J]. *Microbes and Environments*, 2010, 25(1): 45-48
- [32] Enwall K, Throbäck I N, Stenberg M, et al. Soil resources influence spatial patterns of denitrifying communities at scales compatible with land management[J]. *Applied and Environmental Microbiology*, 2010, 76(7): 2243-2250
- [33] Ducey T F, Shriner A D, Hunt P G. Nitrification and denitrification gene abundances in swine wastewater anaerobic lagoons[J]. *Journal of Environmental Quality*, 2011, 40(2): 610-619
- [34] Liu B B, Mørkved P T, Frostegård Å, et al. Denitrification gene pools, transcription and kinetics of NO , N_2O and N_2 production as affected by soil pH[J]. *FEMS Microbiology Ecology*, 2010, 72(3): 407-417
- [35] Billings S A, Tiemann L K. Warming-induced enhancement of soil N_2O efflux linked to distinct response times of genes driving N_2O production and consumption[J]. *Biogeochemistry*, 2014, 119(1/3): 371-386

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv –Machine translation. Verify with original.